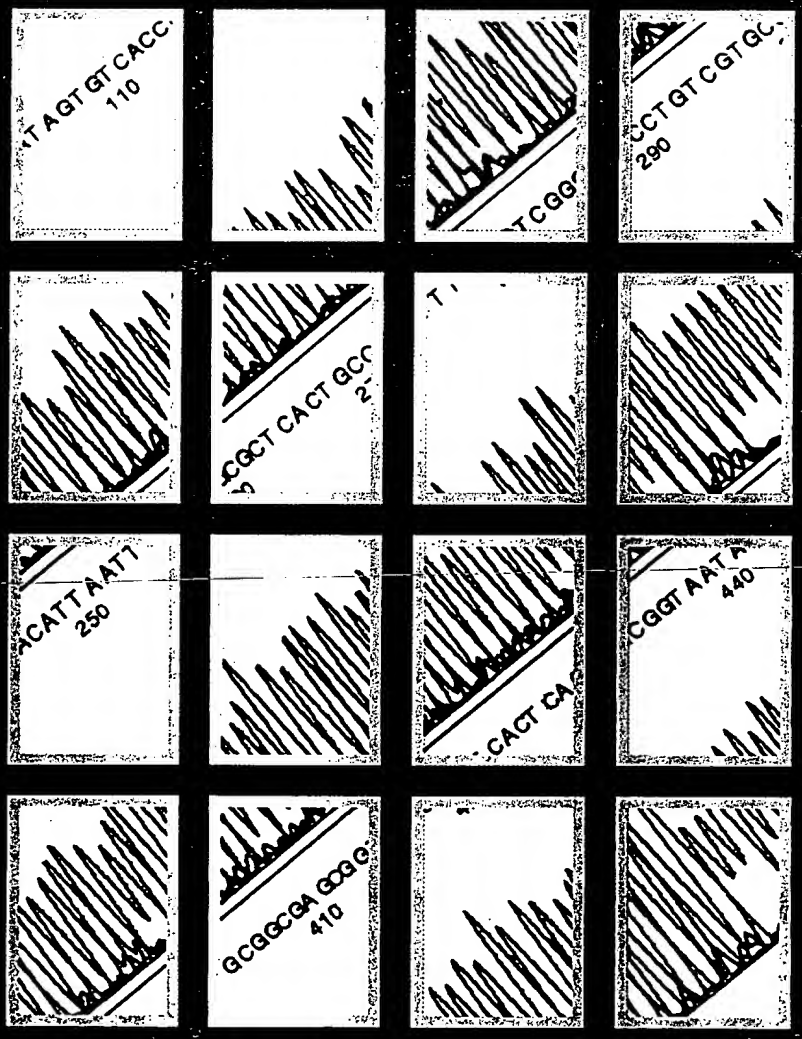
ISSUE 20

LIFE SCIENCE news



^{33}P ddNTPs and Thermo Sequenase for artefact-free DNA sequencing

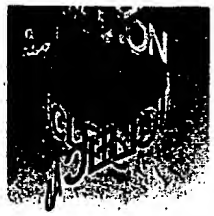
Rapid analysis and economical documentation of electrophoresis gels

SeeDNA - visible aid to DNA precipitation

A nine-point checklist for efficient hybridization

EXHIBIT 9

Amersham LIFE SCIENCE



NUCLEIC ACID LABELLING AND DETECTION

SeeDNA - a visible carrier for more efficient, rapid and reliable nucleic acid precipitation

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seeDNA

Many procedures in molecular biology require the retrieval of small amounts of nucleic acids by alcohol precipitation. Unfortunately, commonly used microgram quantities of nucleic acids are difficult to visualize without high speed centrifugation for long periods of time. Important samples may be reduced to low yields or lost completely, through incomplete precipitation or difficulty in visualizing the tiny, translucent pellet. The use of 'carriers' such as tRNA or glycogen can improve nucleic acid recovery but can also interfere with procedures downstream from precipitation.

To remove the uncertainty associated with alcohol precipitation, Amersham has introduced SeeDNA™ co-precipitant*. This brightly coloured, polymer-based carrier molecule has been designed specifically for nucleic acid precipitation and is suitable for use with DNA and RNA. It offers a number of advantages over traditional carriers used in ethanol precipitation:

- Vivid pink colour ensures easy pellet location (Figure 1).
- Efficient precipitation ensures quantitative nucleic acid recovery.
- Chemically inert, ensures no inhibition of downstream experimental procedures.
- Facilitates recovery of even minute quantities of nucleic acids (less than 2ng/ml).

DNA precipitated using SeeDNA forms tight pellets which are clearly visualized and easily recovered using the simple 5 minute protocol. Pellet solubilization is also easily followed as the SeeDNA dissolves along with the co-precipitated sample.

SeeDNA is chemically inert and has been tested for compatibility with a variety of applications. A comparison of the properties of several carriers is shown (Table 1).

Experiments to determine the size range of recovered product has shown that DNA molecules larger than 68 bases are recovered efficiently with virtually no recovery of molecules below 50 bases. These results indicate that small single and double stranded molecules and unincorporated nucleotides are efficiently removed using SeeDNA.

The excellent recoveries possible when using SeeDNA have been studied (Table 2). Gel analysis (not shown) verified the absence of unincorporated nucleotides in the precipitated material.

Transformation

Transformations can be sensitive to a variety of compounds and often follow a number of precipitation steps. They are also an accurate method of indicating the recovery of minute quantities of DNA. The effect on transformation efficiency of SeeDNA precipitation was compared to a standard ethanol precipitation protocol (Figure 2). As can be seen, the usage of SeeDNA resulted in a 50-fold greater yield of colonies compared to the control sample without carrier.

Sequencing

DNA sequencing protocols, particularly those using double-stranded templates, require precipitation of small amounts of DNA before primer annealing. Low yields or even loss of the pellet during precipitation often cause the sequencing reaction to fail. The use of See DNA during the precipitation step can overcome these problems and has no affect on the sequencing data (Figure 3) obtained from the ™Vistra DNA Sequencer 725.

Limitations

The fluorophore interferes with the rhodamine-labelled primers and terminators used by Applied Biosystems automated sequencers and may also interfere with other methods using rhodamine detection.

In addition, the fluorophore in SeeDNA absorbs in the UV, preventing spectrophotometric determination of DNA and RNA concentrations. However, such procedures usually involve higher nucleic acid concentrations, (>100µg/ml), which are more easily precipitated and visualized in the absence of carriers.

Summary

SeeDNA co-precipitant can greatly improve the results of almost all laboratory procedures where a precipitation step is required. The high visibility of the pellet and the improved sample recoveries can reduce experimental failures due to losses of critical samples.

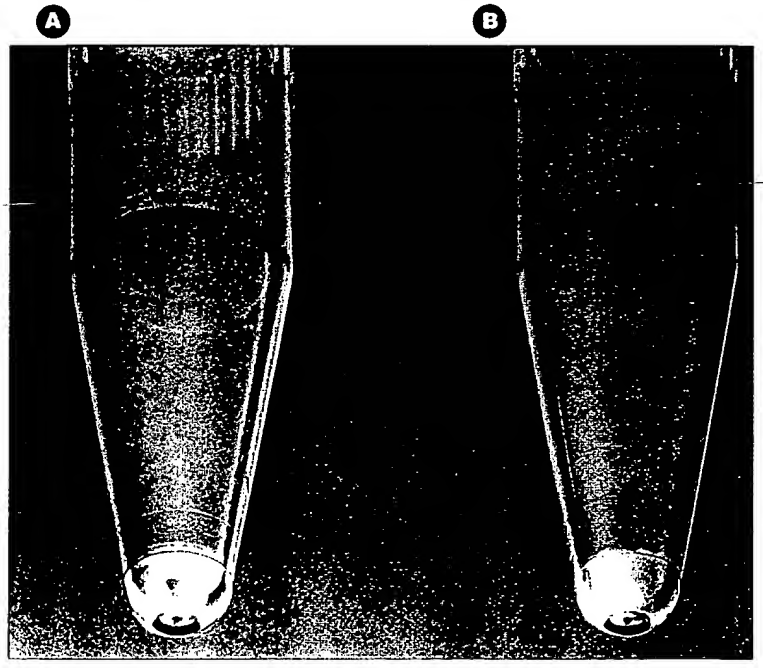

Figure 1 Comparison of precipitated pellet visibility

A Standard ethanol precipitation procedure plus 2µl of SeeDNA co-precipitant.

B Standard ethanol precipitation procedure.

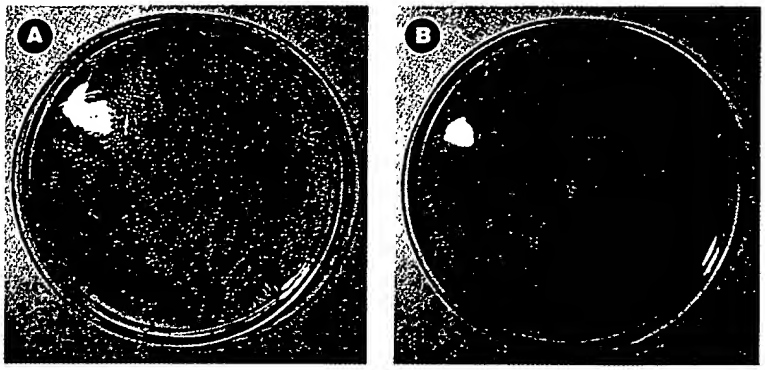

Figure 2 Comparison of transformation efficiency found when using SeeDNA and a standard ethanol precipitation protocol

A 1ng pUC119 precipitated with SeeDNA, transformed into pMOS*Blue* competent cells, cell efficiency $2 \times 10^{-}$ cfu/µg^{-1} pUC119.

B 1ng pUC119 precipitated using standard ethanol precipitation, transformed into pMOS*Blue* competent cells, cell efficiency 4×10^{5} cfu/µg^{-1} pUC119.

Picture taken with the *Kodak Digital Science* Scientific Digital Documentation system (SDDS), available through Amersham (for more information see page 7).

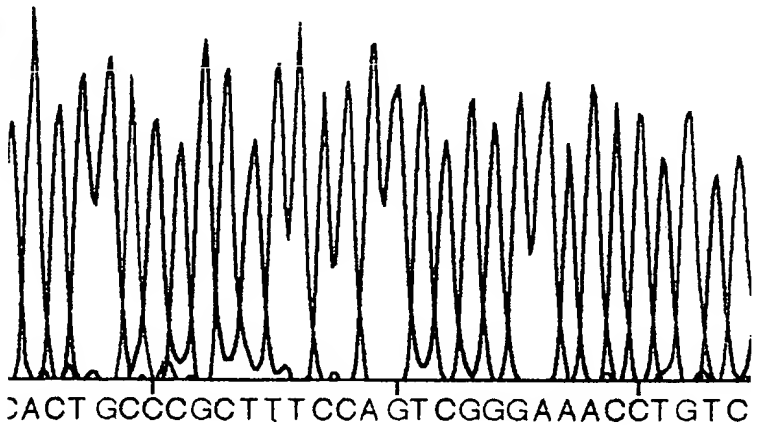

Figure 3 SeeDNA precipitated DNA sequence
mp19 RF precipitated using SeeDNA.

Sequencing reactions prepared using the Thermo Sequenase core sequencing kit (RPN 2440) and Texas Red™ labelled primer.

Sequence generated on the Vistra DNA Sequencer 725.

Table 1 Comparison of different carriers for precipitation of nucleic acids

	SeeDNA	Glycogen	tRNA
Easily visualized	✓	-	-
Free of DNA and RNA	✓	?	-
Compatible with:			
Gel electrophoresis	✓	✓	-
PCR amplification	✓	?	-
DNA sequencing	✓	✓	-
Restriction digestion	✓	✓	✓
Ligation	✓	✓	?
Transformation	✓	?	-
cDNA synthesis	✓	?	-
Kinase reactions	✓	✓	-
Random priming	✓	?	-
In vitro transcription	✓	✓	?
In vivo translation	✓	✓	✓

Table 2 Recovery of various RNA and DNA samples with SeeDNA as carrier
The indicated samples of ^{32}P-labelled RNA and DNA were prepared using standard protocols for *in vitro* transcription and random priming, respectively. Following the labelling reactions, incorporation was determined by DE81 filtration. Known amounts of incorporated material (300,000 cpm) were precipitated in the presence of SeeDNA. Samples without SeeDNA resulted in a 5-50 fold reduction in recovery.

Sample	Incorporated cpm recovered
RNA (100nt, 0.2ng/µl)	90%
RNA (1,000nt, 0.2ng/µl)	92%
RNA (10,000nt, 0.2ng/µl)	89%
DNA (100-2000bp, 4pg/µl)	86%

To receive more information on SeeDNA co-precipitant, circle reader reply 198.